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葱属 *Amerallium* 亚属（石蒜科）的系统发生与性状进化*

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摘要: 运用贝叶斯和简约法对葱属（*Allium*）*Amerallium* 亚属的核糖体 DNA 内转录间隔区（ITS）进行了分析, 对该亚属的系统发生进行了推测。系统分析证实 *Amerallium* 是单系的, 并表明该亚属由三个隔离的地理群组成: 北美 *Ameralliums*, 地中海区 *Ameralliums* 和东亚 *Ameralliums*。性状进化的重建表明鳞茎是原始或祖先状态, 根状茎和肉质增粗的根是衍生状态且在 *Amerallium* 这个亚属的类群中独立进化发生了几次。重建也表明该亚属的原始染色体基数 $x=7$, 其它染色体基数（$x=8, 9, 10, 11$）是由它转化而来的。在北美类群中, 异基数性相当罕见, 而多倍性似乎是一个相对频繁的进化事件。在地中海区类群和东亚类群中, 异基数性和多倍性是染色体进化的两个主要驱动力。

关键词: 葱属; *Amerallium*; 性状进化; ITS; 系统发生
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Phylogeny and Character Evolution in *Allium* Subgenus *Amerallium* (Amaryllidaceae)

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Abstract: Bayesian and parsimony analyses of the nuclear ribosomal DNA internal transcribed spacer (ITS) were used to infer the phylogeny of *Allium* subgenus *Amerallium*. Phylogenetic analyses corroborate that *Amerallium* is monophyletic and the results indicate that *Amerallium* composed of three isolated geographical groups: North American *Ameralliums*, the Mediterranean region *Ameralliums*, and eastern Asian *Ameralliums*. Reconstruction of character evolution suggests bulbs as a primitive or ancestral state, and rhizomes and thick fleshy roots as derived states which have evolved and developed several times independently within the groups of *Amerallium*, and the basic chromosome number $x=7$ is the primitive state and other basic chromosomes numbers ($x=8, 9, 10, 11$) are derived from $x=7$. Within North American *Ameralliums*, dysploidy is a rather rare evolutionary event and polyploidy seems to be a relatively frequent evolutionary event. Within the Mediterranean region and eastern Asian *Ameralliums*, both dysploidy and polyploidy are two primary driving forces in their chromosome evolution.

Key words: *Allium*; *Amerallium*; Character evolution; ITS; Phylogeny

The genus *Allium* L. consists of approximately more than 800 species according to Fritsch *et al.* (2010). To some extent, this is consistent with the current online version of the World Checklist of Selected Plant Families maintained by Royal Botanic Gardens, KEW (UK, http://apps.kew.org/wcsp/reportbuilder.do), which recognizes 881 species. *Allium* is a member of order Asparagales, family

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Amaryllidaceae J.St.-Hil., subfamily Allioideae Herb., tribe Allieae Dumort. (Fay and Chase, 1996; APG, 2009; Chase *et al.*, 2009). After Fay and Chase (1996), Friesen *et al.* (2000) and Chase *et al.* (2009), *Allium* (including *Caloscordum* Herb., *Milula* Prain and *Nectaroscordum* Lindl.) is the only genus in tribe Allieae. Previous molecular data suggested that *Allium* evolution proceeded in three separate evolutionary lines (Fritsch, 2001; Fritsch and Friesen, 2002; Friesen *et al.*, 2006; Li *et al.*, 2010) and *Amerallium* Traub is the largest subgenus in the most ancient line comprising around 135 species occurring in North America (New World), the Mediterranean region and eastern Asia (Old World) (Traub, 1968; Friesen *et al.*, 2006). Members of this subgenus are extremely diverse in ecology and grow in dry stony slopes, Mediterranean garigues, cliffs, river banks, prairies, mountains and subalpine meadows (Hanelt *et al.*, 1992). The subgenus is very diverse in cytology and contains all the basic chromosome numbers in the genus *Allium*. The most common basic chromosome number is $x=7$, and yet other numbers ($x=8$, 9, 10, 11) also exist (Traub, 1968; Sen, 1974; Yan *et al.*, 1990; Huang *et al.*, 1995; Huang *et al.*, 1996a, b; Xu *et al.*, 1998; Ni, 1999; Xu and Kamelin, 2000; Dale *et al.*, 2002; Zhang and Xu, 2002; Zhang *et al.*, 2008, 2009; Wei *et al.*, 2011).

Historically, Traub (1968, 1972) proposed classifying the 600 or so *Allium* species under three subgenera: *Allium* L., *Amerallium* and *Nectaroscordum* (Lindl.) Asch. et Graebn. In Traub's classification, *Allium siculum* Ucria was the type species of subgenus *Nectaroscordum*; subgenus *Amerallium* united the North American species with the Mediterranean *Alliums* classified under section *Molium* Endl. and species that are now classified under sections *Arctoprason* Kirschl. and *Briseis* (Salisb.) Stearn (Hanelt *et al.*, 1992); and the rest of the species were lumped together in subgenus *Allium*. Based on a multidisciplinary approach, Hanelt *et al.* (1992) reassembled the species pooled by Traub in subgenus *Allium* into six subgenera namely *Allium*, *Amerallium*, *Bromatorhiza* Ekberg, *Caloscordum* (Herb.) R. M. Fritsch, *Melanocrommyum* (Webb & Berth.) Rouy and *Rhizirideum* (G. Don ex Koch) Wendelbo, while excluded *Nectaroscordum* from classifications of the genus *Allium*. Recently, molecular approaches such as chloroplast DNA and nuclear ribosomal DNA (nrDNA) have been applied to understand the evolutionary processes and taxonomic relations within the genus *Allium* and also in subgenus *Amerallium*. A first approach to structuring the genus *Allium* itself by molecular markers was published by Linne von Berg *et al.* (1996). The resulting phenogram largely confirmed the subgeneric classification based on an integration of morphological and other methods, but found that subgenus *Amerallium* and *Bromatorrhiza* could not be clearly distinguished. The subgenus *Bromatorrhiza* (including section *Bromatorrhiza* Ekberg, *Coleoblastus* Ekberg, *Cyathophora* R. M. Fritsch), originally circumscribed by Ekberg (1969) by the presence of fleshy roots as storage organs and the lack of true storage bulbs or rhizomes, again proved to be paraphyletic and had to be cancelled in other studies (Samoylov *et al.*, 1995, 1999; Mes *et al.*, 1997, 1999; Friesen *et al.*, 2000). Later, Friesen *et al.* (2006) presented a new classification of genus *Allium* consisting of 15 subgenera (including *Nectaroscordum*) based on their phylogenetic study, in which they confirmed the artificial character of subgenus *Bromatorrhiza* and placed section *Bromatorrhiza* in subgenus *Amerallium* and other two sections in subgenus *Cyathophora* (R. M. Fritsch) R. M. Fritsch. Li *et al.* (2010) again corroborated the artificial character of subgenus *Bromatorrhiza* and agreed with their taxonomic treatment. The distribution of *Amerallium* species between Old World and New World was well reflected in the phylogenetic data (Dubouzet and Shinoda, 1999; Friesen *et al.*, 2006; Nguyen *et al.*, 2008; Li *et al.*, 2010), and the origin and migration about *Amerallium* has been long in dispute. Hanelt *et al.* (1992) postulated that the *Ameralliums*

had its origins in Asia and spread to North America via the Bering Land Bridge, but they did not discuss the origins of the Mediterranean *Amerallliums*. The alternate hypothesis is a predominantly unidirectional migration via the land bridges at Bering and North Atlantic (Dubouzet and Shinoda, 1999). Nguyen *et al.* (2008) investigated the evolutionary history of *Alliums* in western North America (especially in California) and their adaptation to serpentine soils. Their results also represent a first attempt at initiating a more detailed study on the biogeography of subgenus *Amerallium* in North America and imply that two separate biogeographic patterns led to *Amerallium* diversification in North America. Based on those researches, Li *et al.* (2010) further examined the migration route of the *Amerallliums* by the inclusion of species endemic to eastern Asia that have often been excluded from previous analyses. They proposed that the ancestor of *Amerallium* originated in eastern Asia and one lineage of *Amerallium* likely spread eastward to North America via the Bering Land Bridges and expanded their range southward, while the other lineage of *Amerallium* expanded its range from east to west and ended up in the Mediterranean region, not across the North Atlantic.

All the above-mentioned works have been useful in understanding of *Amerallium* phylogeny and biogeography, but the diversity of cytological data and other character have not been tested in a phylogenetic framework to date. In order to better understand the character evolution of *Amerallium*, species endemic to eastern Asia were again incorporated in the present study, but we still lack samples of the Mediterranean *Amerallium* which are also poorly sampled in the previous studies. Nevertheless, it seems appropriate now to publish the results of our study, partly because these are important for considerations of phylogeny and character evolution of *Amerallium* and partly because we hope our results will stimulate further studies. The goals of the present study were to: (1) construct phylogenetic relationships within *Amerallium*; (2) elucidate possible patterns of underground storage organs and chromosome numbers evolution in *Amerallium* under the phylogenetic framework.

1 Materials and methods

1.1 Taxon sampling

Our sampling scheme was designed to cover those taxonomic and geographic *Amerallium* groups, and 64 taxa from North America (47 out of about 81 species), the Mediterranean region (10 out of about 46 species), and eastern Asia (18 samples, representing 5 species and 2 varieties out of about 8 species) were included in the present study. *Allium bulgaricum* (Janka) Prodán, *A. siculum* Ucria, and *A. monanthum* Maxim., were designated as outgroups according to previous studies (Fritsch, 1988, 2001; Fritsch and Friesen, 2002; Friesen *et al.*, 2006; Nguyen *et al.*, 2008; Li *et al.*, 2010). The sources of ITS sequences obtained from original materials and GenBank accession numbers for all other species included in this investigation are listed in the Appendix 1. All accessions in the collection stem from populations collected during field trips. Voucher specimens were deposited in the herbarium of the Sichuan University (SZ).

1.2 DNA extraction, amplification and sequencing

Genomic DNA was extracted from silica gel-dried or fresh leaves by using the method of Doyle and Doyle (1987). The ITS region was amplified with primers ITS4 and ITS5 (White *et al.*, 1990). The PCR parameters were as follows: 94℃ for 5 min; 30 cycles of 94℃ for 45 s, 55℃ for 45 s, 72℃ for 1 min; and 72℃ for 7 min. PCR products were separated by 1.5% (w/v) agarose TAE gel and purified using Wizard PCR preps DNA Purification System (Promega, Madison, WI, USA) following manufacturer's instructions. The purified PCR products were analyzed in an ABI 310 Genetic Analyzer (Applied Biosystems Inc.) in both directions using the PCR primers.

1.3 Sequence comparisons and phylogenetic analyses

DNA sequences were initially aligned using the default pairwise and multiple alignment parameters

in Clustal X (Jeanmougin *et al.*, 1998) and then rechecked and adjusted manually as necessary using MEGA 4 (Tamura *et al.*, 2007). Gaps were positioned to minimize nucleotide mismatches and treated as missing data in phylogenetic analyses.

Phylogenetic analyses were conducted by employing maximum parsimony (MP) criteria and Bayesian Inference (BI), using the programs PAUP* version 4.0b10 (Swofford, 2003) and MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003), respectively. For MP, heuristic searches were carried with 1 000 random addition sequence replicates. One tree was saved at each step during stepwise addition, and tree-bisection-reconnection (TBR) was used to swap branches, and the maximum number of trees was set to 10 000. All characters were unordered and equally weighted. Gaps were treated as missing data. Bootstrap values were calculated from 1 000 000 replicate analyses using "fast" stepwise-addition of taxa and only those values compatible with the majority-rule consensus tree were recorded. Prior to a Bayesian analysis, MrModeltest version 2.2 (Nylander, 2004) was used to select a best-fit model of nucleotide substitution, and the GTR+I+G model under the AIC was selected. The Bayesian Markov Chain Monte Carlo (MCMC) algorithm was run for 2 000 000 generations with one cold chain and three heated chains, starting from random trees and sampling trees every 100 generations. The first 5 000 trees were considered as the burn-in and discarded. A 50% majority-rule consensus tree of the remaining trees was produced.

1.4 Estimation of ancestral character states

Selected characters, namely underground storage organs and chromosome numbers, were defined according to previous studies of these *Alliums* (Traub, 1968; Sen, 1974; Pastor and Valdés, 1988; Tzanoudakis and Vosa, 1988; Yan *et al.*, 1990; Huang *et al.*, 1995; Huang *et al.*, 1996a, b; Ohri *et al.*, 1998; Xu *et al.*, 1998; Ni, 1999; Xu and Kamelin, 2000; Dale *et al.*, 2002; Zhang and Xu, 2002; Ricroch *et al.*, 2005; Friesen *et al.*, 2006; Zhang *et al.*, 2008, 2009; Wei *et al.*, 2011). Three states were chosen for underground storage organs: bulbs (0), rhizomes (1), and thick fleshy roots (2), and five states were chosen for basic chromosome numbers: 7 (0), 8 (1), 9 (2), 10 (3) and 11 (4). To infer ancestral character states, Parsimony optimizations were performed in the software Mesquite v. 2.01. (Maddison and Maddison, 2007). Considering all accessions of the same species composed a well-supported clade, a reduced taxonomic subset was obtained and then phylogenetic analysis was conducted by employing BI with the methods described above. Optimizations were run on the 50% majority rule tree from Bayesian analysis and the character states were treated as "unordered" (i.e., allow free transformation of a character state to any other states).

2 Results

2.1 Sequence analyses

The ITS region varied in length from 589 bp (*A. shevockii* McNeal) to 661 bp (*A. hoffmanii* Ownbey ex Traub). After introducing the necessary gaps, the ITS alignment was 706 bp in length and resulted in 225 constant characters and 463 variable characters of which 390 were parsimony-informative. The mean GC content of the ITS region was 52.5%.

2.2 Phylogenetic analyses

Trees inferred from Bayesian analysis and maximum parsimony showed no significant difference in their topologies, therefore here the Bayesian tree with posterior probabilities (PP) and bootstrap support values (BS) is shown in Fig. 1. In all analyses, the subgenus *Amerallium* proved to be monophyletic. Within subgenus *Amerallium*, the New World *Amerallium* clade is sister to the Old World *Amerallium* clade (PP=1.00, BS=99%). The New World *Amerallium* clade (PP=1.00, BS=97%) contains two groups. One group (PP=0.55, BS=53%) includes several subclades corresponding to sections *Amerallium* Traub+*Caulorhizideum* Traub+*Rhopetoprason* Traub with species native to mid-western and

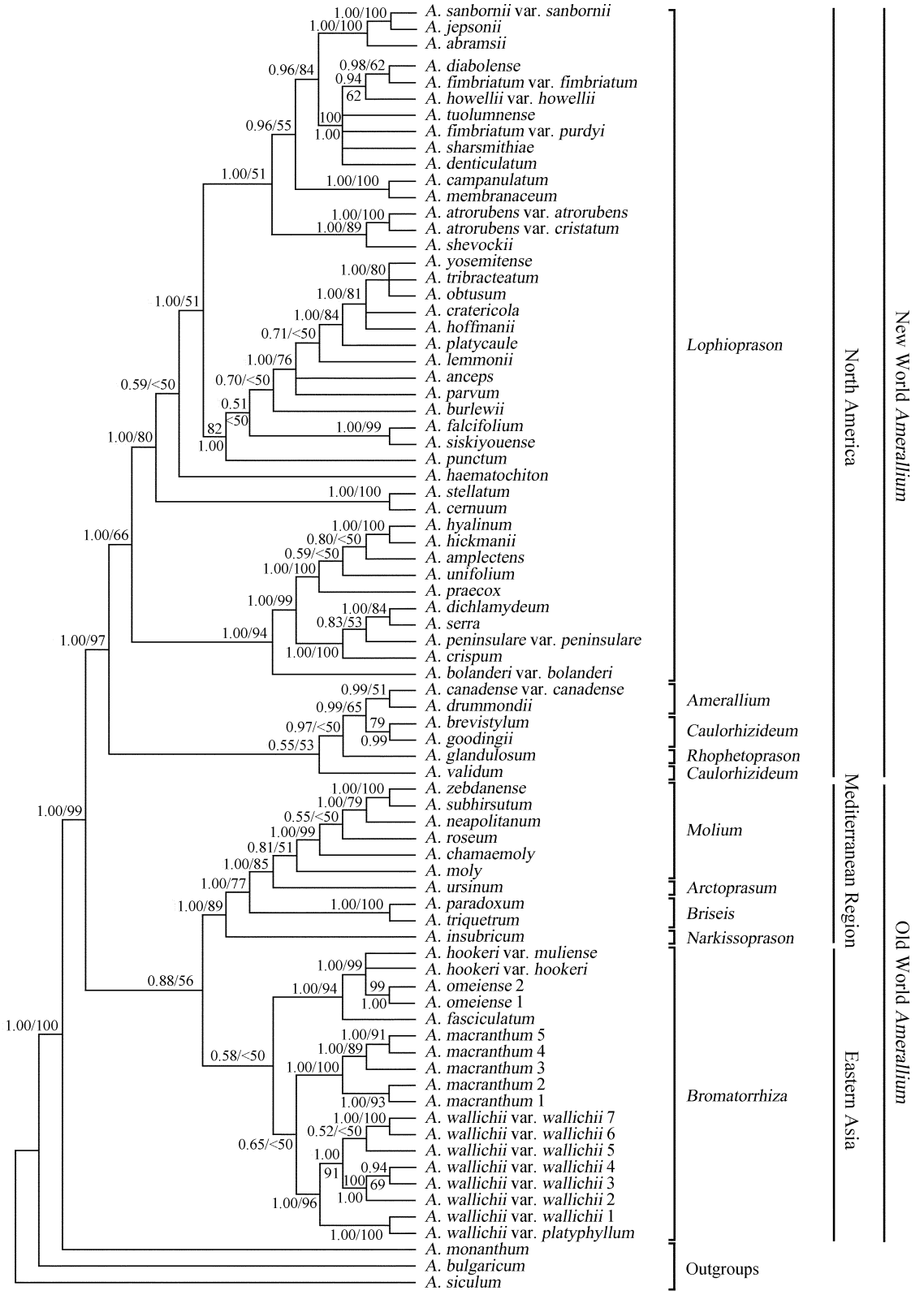

Fig. 1　Phylogenetic tree resulting from a Bayesian analysis of the ITS sequences from species of *Amerallium* and three outgroup species. The subgeneric and sectional classification according to Hanelt *et al.* (1992), Dubouzet and Shinoda (1999), Friesen *et al.* (2006), Nguyen *et al.* (2008), and Li *et al.* (2010) is indicated on the right. Values along branches represent Bayesian posterior probabilities (PP) and parsimony bootstrap (BS), respectively

southwestern United States (with few exceptions, e. g., *A. validum* S. Watson). The other group (PP = 1.00, BS = 66%) includes monophyletic section *Lophioprason* Traub with species restricted to western North America (the only exceptions are *A. cernuum* Roth and *A. stellatum* Ker Gawler). Within the Old World *Amerallium* clade (PP = 0.88, BS = 56%), two sister subclades are evident: one with species from the Mediterranean region; the other with species from the Himalayas and South-West China. In the Mediterranean subclade, section *Narkissoprason* Kam. is sister to a clade containing sections *Briseis* (Salisb.) Stearn, *Arctoprasum* Kirschl., and *Molium* G. Don ex Koch (PP = 1.00, BS = 89%). In the Himalayas and South-West China clade, *A. wallichii* var. *wallichii* Kunth, *A. wallichii* var. *platyphyllum* (Diels) J. M. Xu and *A. macranthum* Baker are sister to a clade comprising *A. omeiense* Z. Y. Zhu, *A. fasciculatum* Rendle, *A. hookeri* Thwaites var. *hookeri* and *A. hookeri* var. *muliense* Airy Shaw (PP = 0.58, BS < 50%). The five accessions of *A. macranthum* composed a well-supported clade (PP = 1.00, BS = 100%) that was sister to a clade containing seven accessions of *A. wallichii* var. *wallichii* and one of *A. wallichii* var. *platyphyllum* (PP = 1.00, BS = 96%), and these two clades constituted a weakly supported clade (PP = 0.65, BS < 50%). *A. omeiense*, *A. hookeri* var. *hookeri* and *A. hookeri* var. *muliense* form a trichotomy (PP = 1.00, BS = 99%) and this trichotomy is sister to *A. fasciculatum* (PP = 1.00, BS = 94%). the two accessions of *A. omeiense* formed a strongly supported clade (PP = 1.00, BS = 99%).

2.3 Character state reconstruction

Two morphological characters, underground storage organs and basic chromosome numbers were optimized onto the 50% majority rule tree. Parsimonious optimization suggested that bulbs are primitive, and rhizomes and thick fleshy roots are derived states within *Amerallium* (Fig. 2A). Optimization of chromosome numbers suggested $x = 7$ to be the ancestral basic chromosome number and the chromosome number in *Amerallium* evolved from $x = 7$ to other basic numbers (Fig. 2B).

3 Discussion

3.1 Phylogeny within the subgenus *Amerallium*

Amerallium spp. are extremely diverse in morphology, in which some produce mainly rhizomes and poorly developed bulbs and others form distinct bulbs and broad leaves similar to those common in the subgenus *Melanocrommyum*, or very narrow leaves as in the subgenus *Allium* (Kamenetsky and Rabinowitch, 2006). However, morphological synapomorphies for *Amerallium* include one row of vascular bundles, absence of palisade parenchyma and subepidermal position of laticifers (Traub, 1968, 1972; Fritsch, 1988). Furthermore, strong serological affinities and the dominating basic chromosome number of $x = 7$ strongly support its separate status. The results presented here continue to support earlier finding that *Amerallium* is monophyletic (Samoylov *et al.*, 1995; Dubouzet and Shinoda, 1999; Friesen *et al.*, 2006; Nguyen *et al.*, 2008; Li *et al.*, 2010). In accordance with studies of Dubouzet and Shinoda (1999), our molecular data underline the existence of two distinct biogeographic clades, namely the Old World clade and the New World clade. Both clades are a monophyletic unit, which agrees with a uniform electrophoretic banding pattern of salt-soluble seed storage proteins (Maass, 1992). Furthermore, our results indicate that *Amerallium* composed of three isolated geographical groups: one comprising almost all *Allium* species native to North America (New World) and the remainder containing two smaller groups from the Mediterranean region and eastern Asia (Old World). In the well-supported North American *Amerallium* clade, the sister relationship of section *Lophioprason* to sections *Amerallium* + *Caulorhizideum* + *Rhopetoprason* is well supported. *Allium validum* from section *Caulorhizideum* is sister to a clade containing the remaining *Caulorhizideum* + *Amerallium* + *Rhopetoprason*. Thus, section *Caulorhizideum* is found to be non-monophyletic. Section *Lophioprason* which comprises species that

are native to California or restricted to western North America in their distributions was found to be monophyletic. Within the Old World *Amerallium* clade, the monophyletic section *Bromatorrhiza* Ekberg was sister to a clade containing all other Mediterranean region sections. In the Mediterranean region taxa the species of section *Molium* show greater affinity to each other than to those of other sections including *Narkissoprason*, *Briseis* and *Arctoprasum*. *Allium ursinum*, while sister to the *Molium*, is maintained as a separate monotypic section *Arctoprasum*, which is also supported by various unique characteristics such as leaf morphology and anatomy, leaf sequence, bulb morphology, secondarily reduced ovule number and size, seedling morphology, cpDNA variability (Pastor and Valdés, 1985; Fritsch, 1988; Druselmann, 1992; Hanelt *et al.*, 1992; Kruse, 1992; Samoylov *et al.*, 1995). Section *Briseis* was isolated from *Molium* on the basis of distinctive structure of filaments and style and the presence of elaiosomes on the seeds (Stearn, 1946) and their relationship is also reflected in our phylogenetic studies. According to Li *et al.* (2010) and our present studies, within *Bromatorrhiza*, two sister groups are evident, one with species *A. wallichii* var. *wallichii*, *A. wallichii* var. *platyphyllum* and *A. macranthum*, and the other with species *A. omeiense*, *A. guanxianense* J. M. Xu, *A. xiangchengense* J. M. Xu, *A. hookeri* var. *hookeri* and *A. hookeri* var. *muliense*, *A. fasciculatum*, *A. chienchuanense* J. M. Xu.

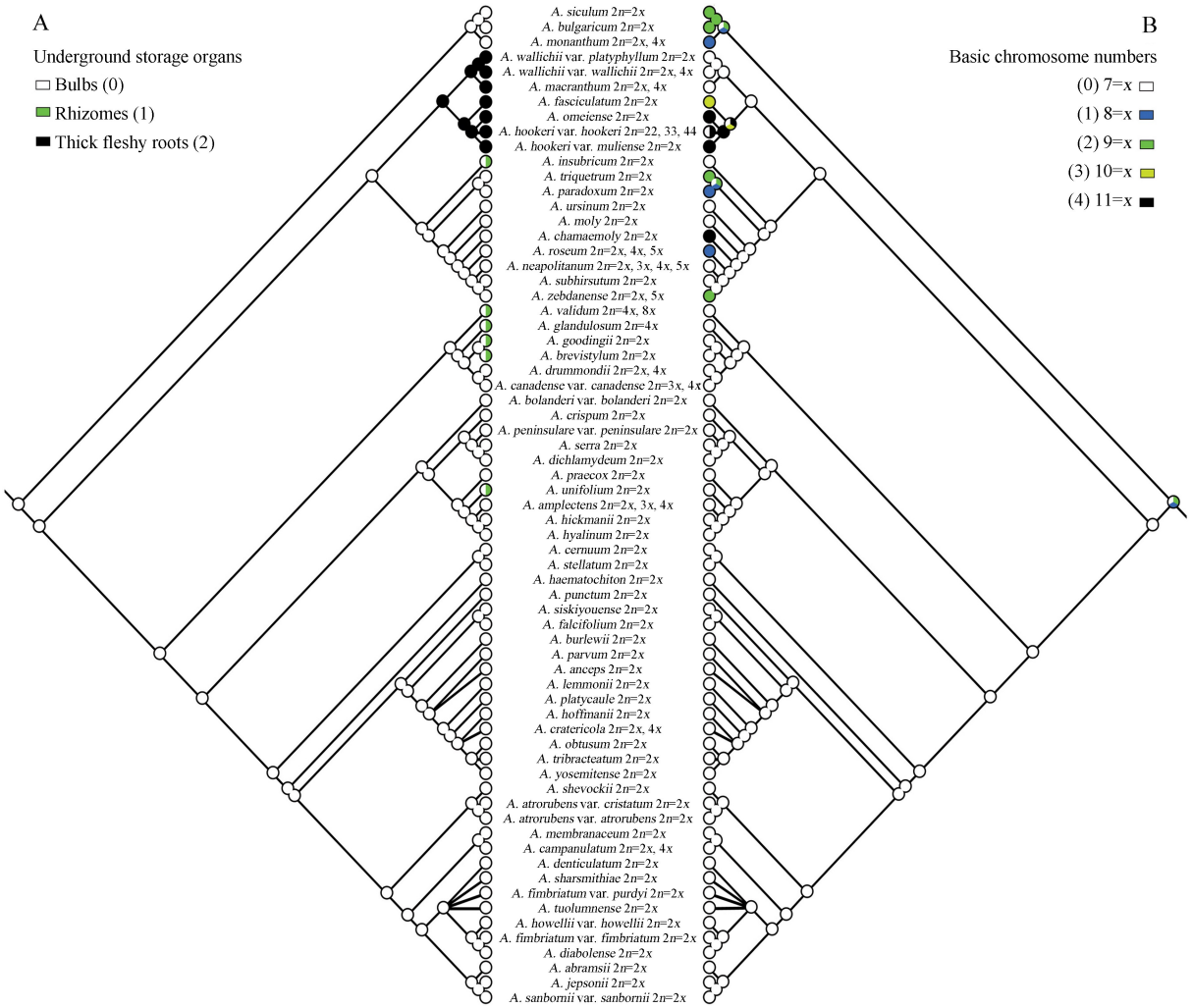

Fig. 2　Evolution of categorical characters on the Bayesian topology: A, underground storage organs (bulbs, rhizomes, and thick fleshy roots); B, basic chromosome numbers (7, 8, 9, 10, and 11). Colours are explained in the legend of each figure

3.2 Character evolution in *Amerallium*

3.2.1 Underground storage organs

Within the New World (North America) *Amerallium*, two sections, *Caulorhizideum* and *Rhophetoprason* with rhizomes and bulbs developed have a southernmost distribution in the New World in Mexico and Guatemala whereas sections with true bulbs (*Amerallium* and *Lophioprason*) occur mainly in mountains of western North America (Hanelt *et al.*, 1992; Pistrick, 1992; Ohri *et al.*, 1998). In the Mediterranean region *Ameralliums*, except for section *Narkissoprason*, which also has rhizomes, all the extra-Mediterranean sections have true bulbs. Bulbs are dominant in these two regions, which may represent an adaptation to environments with a Mediterranean type climate characterized by hot and dry summers, but cool and moist winters favourable for plant growth and development (McNeal and Ownbey, 1973; Hanelt, 1990). Bulbs can float unimpaired for a long time in salt water (De Wilde-Duyfjes, 1976; Stearn, 1978), which may also explain the predominantly coastal distribution of Mediterranean *Moliums* (Dubouzet and Shinoda, 1999). Section *Bromatorrhiza* is a small insufficiently studied group occurring in ecologically mesophytic high mountain regions of western Himalaya and south-west China as a component of moist grassy slopes, rocks, and herb layer of forests (Hanelt *et al.*, 1992). The special character of this group differing from the other sections in the *Amerallium* is the presence of thick fleshy roots as storage organs, without true bulbs or rhizomes, and this special character also exists in sections *Coleoblastus* Ekberg and *Cyathophora* R. M. Fritsch which belong to subgenus *Cyathophora* (R. M. Fritsch) R. M. Fritsch. Previous studies (Fritsch and Friesen, 2002; Friesen *et al.*, 2006; Li *et al.*, 2010) have indicated that subgenus *Cyathophora* is one member of the third evolutionary line in *Allium* evolution. This may suggest the process of convergent evolution, in which those two distinct lineages evolve a similar characteristic (thick fleshy roots) independently of one another. The reason may be that both lineages distributed in the Himalaya and south-west china face similar environmental challenges and selective pressures. Parsimonious optimization suggests bulbs as a primitive or ancestral state, and rhizomes and thick fleshy roots as derived states which have evolved and developed several times independently within the groups of *Amerallium* (Fig. 2A).

3.2.2 Chromosome numbers

Former researchers have different views about the evolution of basic chromosome numbers in the genus *Allium*. Levan (1932, 1935) has suggested their origin in the form of an ascending series. Mensinki (1940) has also put forward an alternative view according to which the basic numbers seven and nine have both arisen from eight. Brat (1965) considered that the basic numbers in different taxonomic groups of the genus *Allium* have arisen in independent orders. Previous molecular studies have indicated that *Allium* is monophyletic (Friesen *et al.*, 2006; Nguyen *et al.*, 2008; Li *et al.*, 2010), so there should be a single primitive basic chromosome number for the entire genus although the primitive basic chromosome number may vary in different subgenera. It is regrettable that one cannot, at present, deduce with confidence the primitive state of the basic chromosome number and the direction of the basic chromosome numbers changes within the phylogeny of the entire *Allium* spp. We just infer the primitive basic chromosome number and the direction of the basic chromosome number changes within the subgenus *Amerallium* and the primitive basic chromosome number of the entire *Allium* is out of the scope of the present paper.

In the subgenus *Amerallium*, most species are diploid with basic chromosome numbers of $x=7$, 8, 9, 10 or 11; $x=7$ being the most common. In addition to these differences in basic numbers, polyploidy has proceeded on four basic series, 7, 8, 9 and 11. The reconstruction of ancestral state in the present study suggested $x=7$ being ancestral and other basic numbers ($x=8$, 9, 10, 11) being de-

rived (Fig. 2B). Levan (1932, 1935) found arm-length asymmetry is more pronounced in the "16" and "18" -chromosomes types and took this to mean that the "14" -chromosome types were the most primitive and that the "16" and "18" -chromosome types were derived from them. In subgenus *Amerallium*, symmetrical chromosomes with median-submedian centromeres are most common in species with $x=7$, while species with $x=8$ have usually varying numbers of asymmetrical chromosomes and species with $x=9$, 10, 11 have entirely asymmetrical chromosomes with telocentric chromosomes (Brat, 1965; Huang *et al.*, 1995; Xu *et al.*, 1998). According to the general principles (Stebbins, 1971; Hong, 1990) and minimum interaction hypothesis (Imai *et al.*, 1986; Schubert, 2007) of karyotype evolution, karyotype evolution in higher plants generally tends to develop from symmetry to asymmetry and tends towards an increasing number of acrocentric chromosomes, thereby minimising the risk of deleterious rearrangements, while the opposite tendency, the reduction of chromosome number and formation of metacentric chromosomes, is considered to be the result of 'rare back-eddies' that are generated at random and tolerated or even favoured when they provide short-term advantages. Based on the cytological and molecular data, we propose that, in the subgenus *Amerallium*, the basic chromosome number $x=7$ is the primitive state and other basic chromosomes number ($x=8$, 9, 10, 11) are derived from it. The New World (North America) *Amerallium* clade displays a uniform basic chromosome number with $x=7$. Within the 47 species investigated, 40 species show a noteworthy constancy of chromosome number represented by diploid populations only ($2x$), and four species (*A. drummondii*, *A. amplectens*, *A. cratericola*, *A. campanulatum*) contain both diploid and polyploid ($2x$, $3x$, or $4x$) and three species (*A. canadense* var. *canadense* L., *A. glandulosum* Link & Otto and *A. validum*) exclusively polyploid populations ($3x$, $4x$ or $8x$). So, the basic chromosome number found so far is very stable and no linking dysploid numbers are known within North American *Amerallium*s, which indicate that dysploidy is a rather rare evolutionary event in this group. Compared with dysploidy, polyploidy originated via autoploidization or alloploidization occurs in several North American *Amerallium*s and thus seems to be a relatively frequent evolutionary event. Chromosome structure, such as inversions and translocations, seems to have acted as an important cytogenetic mechanism in the evolution of the North American *Amerallium*, considering that 66 of the 81 *Amerallium* species, i. e. 81.48%, are represented in North America by diploid populations only (data from the statistical results of the North American Flora for *Allium*). Considering all the available chromosomal data, Mediterranean region *Amerallium*s possess the abundant diversity for basic chromosome numbers ($x=7$, 8, 9, and 11) in subgenus *Amerallium*, in which sections *Narkissoprason* and *Arctoprason* with $x=7$, section *Briseis* with $x=7$, 8, 9, and section *Molium* with $x=7$, 8, 9, and 11. The high karyotypic diversity encountered in *Briseis* and *Molium* might indicate rapid evolutionary episodes within those two groups. Furthermore, polyploidy has taken place in the three series of basic chromosome numbers, 7, 8 and 9. Thus, dysploidy and polyploidy seem to have acted as important cytogenetic mechanisms in the evolution of the Mediterranean region *Amerallium*s. Karyotype reconstruction suggests $x=7$ as a possible ancestral basic number for Mediterranean region *Amerallium*s and thus an ascending dysploid series for this group. Yet, the mechanism of the dysploidy is still not well understood. Meiosis involving irregular segregation, unequal translocation, and centric fission are all possible causes of dysploid variations (Stebbins, 1971). Karyotype reconstruction indicates $x=7$ being ancestral for sections *Narkissoprason*, *Arctoprason*, and *Molium*. For *Briseis*, species with $x=7$ are not included in the present study, so karyotype reconstruction did not provide any valuable information for the primitive basic number in this section. Overall, relatively poor rep-

resentatives in this region, do not allow us to propose an unambiguous and credible scenario for karyotype evolution within every section. Detailed sampling of Mediterranean region taxa would be necessary to elucidate the potential scenario. Section *Bromatorrhiza* is a small group restricted to the Himalayas and South-West China and reveals three different basic numbers ($x=7$, 10 and 11), in which two species and one variety (*A. wallichii*, *A. wallichii* var. *platyphyllum* and *A. macranthum*) with $x=7$, one species (*A. fasciculatum*) with $x=10$, and four species and one variety (*A. guanxianense*, *A. xiangchengense*, *A. chienchuanense*, *A. omeiense* and *A. hookeri* var. *muliense*) with $x=11$. Basic numbers for *A. hookeri* var. *hookeri* is complex. Except for few cytotypes recorded to have 33 (Huang *et al.*, 1996b; Zhang and Xu, 2002; Wei *et al.*, 2011) and 44 chromosomes (Yan *et al.*, 1990; Huang *et al.*, 1996b), twenty-two is the most common number on record for this taxon (Sen, 1974; Yan *et al.*, 1990; Huang *et al.*, 1996b; Zhang and Xu, 2002). According to existing researches, $2n=22$ being off-types (trisomics) of segmental allotriploids with $x=7$ (Sharma *et al.*, 2011), $2n=33$ being triploid with $x=11$ (Huang *et al.*, 1996b; Zhang and Xu, 2002; Wei *et al.*, 2011), and $2n=44$ being autoallohexaploid with $x=7$ (Yan *et al.*, 1990) and tetraploid with $x=11$ (Huang *et al.*, 1996b). Four ploidy levels ($2x$, $3x$, $4x$ and $6x$) exist in this group, in which *A. wallichii* var. *wallichii* and *A. macranthum* are diploid and tetraploid, *A. hookeri* var. *hookeri* is allotriploid, triploid, tetraploid and autoallohexaploid, and the remaining species contain diploids only. It is evident that *A. hookeri* var. *hookeri* has significant karyotype differentiation, and detailed cytogenetic study for this taxon is necessary for determining the ploidy level, type of ploidy and its mechanism. Therefore, both dysploidy and polyploidy are two primary driving forces in chromosome evolution of *Bromatorrhiza*. Geological history, unique ecological environment, and micro-environmental diversity in this region could be reasons for the diversity of basic chromosome number. Optimization of the chromosomal data onto the molecular phylogenies reveals $x=7$ to be the most likely ancestral type in *Bromatorrhiza* with other basic numbers being derived mainly through ascending dysploidy (Fig. 2B). The probable mechanism involved in the dysploid differentiation of chromosome numbers in *Bromatorrhiza* is chromosome fission and the subsequent loss of chromosomes.

4 Conclusions

Our present paper has provided insights into the phylogeny and character evolution of *Amerallium*. Despite relatively lack samples of the Mediterranean region *Amerallium*s, we have established evolutionary patterns of underground storage organs and basic chromosome numbers. However, a more detailed phylogenetic study including a larger sample of species, especially the Mediterranean region *Amerallium*s and additional molecular markers and further studies focusing on their morphological characters, will be required to clarify the phylogeny and character evolution of *Amerallium*.

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Appendix 1

Taxa, references, and GenBank accession numbers for all ITS sequences used in the present study. Sequences in bold are our own accessions. [1]Dubouzet and Shinoda (1998); [2]Dubouzet and Shinoda (1999); [3]Friesen *et al*. (2000); [4]Ricroch *et al*. (2005); [5]Friesen *et al*. (2006); [6]Nguyen *et al*. (2008). Subgenus *Amerallium* Traub: *A. abramsii* (Ownbey & Aase) McNeal EU096131[6]; *A. amplectens* Torr. AF055097[2]; *A. anceps* Kellogg EU096134[6]; *A. atrorubens* S. Watson var. *atrorubens* EU096137[6]; *A. atrorubens* var. *cristatum* (S. Watson) McNeal EU096138[6]; *A. bolanderi* S. Watson var. *bolanderi* EU096139[6]; *A. brevistylum* S. Watson AJ412763[5]; *A. burlewii* Davidson EU096142[6]; *A. campanulatum* S. Watson EU096143[6]; *A. canadense* L. var. *canadense* EU096145[6]; *A. cernuum* Roth AF037622[1]; *A. chamaemoly* L. AF055109[2]; *A. cratericola* Eastw. EU096146[6]; *A. crispum* Greene EU096147[6]; *A. denticulatum* (Ownbey & Aase ex Traub) McNeal EU096149[6]; *A. diabolense* (Ownbey & Aase) McNeal EU096150[6]; *A. dichlamydeum* Greene EU096151[6]; *A. drummondii* Regel AJ411908[5]; *A. falcifolium* Hook. & Arn. EU096153[6]; *A. fimbriatum* S. Watson var. *fimbriatum* EU096155[6]; *A. fimbriatum* var. *purdyi* (Eastw.) McNeal EU096156[6]; *A. fasciculatum* Rendle—**GQ181068**; Dazi, Xizang, China; Tu Y-L *et al*. 94-9; *A. glandulosum* Link & Otto AJ412746[5]; *A. goodingii* Ownbey AF055095[2]; *A. haematochiton* S. Watson EU096157[6]; *A. hickmanii* Eastw. EU096159[6]; *A. hoffmanii* Ownbey EU096160[6]; *A. hookeri* Thwaites var. *hookeri* AJ412740[5]; *A. hookeri* var. *muliense* Airy Shaw—**GQ181071**; Xianggelila, Yunnan, China; Xu J-M 93 -25; *A. howellii* Eastw. var. *howellii* EU096161[6]; *A. hyalinum* Curran EU096162[6]; *A. insubricum* Boiss. & Reut. AJ250291[3]; *A. jepsonii* (Ownbey & Aase) S. S. Denison & McNeal EU096163[6]; *A. lemmonii* S. Watson EU096164[6]; *A. macranthum* Baker 1—**HQ690254**; Daocheng, Sichuan, China; Li Q-Q 092303; *A. macranthum* Baker 2—**HQ690256**; Mangkang, Xizang, China; Gao Y-D G2010081702; *A. macranthum* Baker 3—**HQ690255**; Litang, Sichuan, China; Li Q-Q 092206; *A. macranthum* Baker 4—**GQ181072**; Xianggelila, Yunnan, China; Xu J-M 93-23; *A. macranthum* Baker 5—**HQ690562**; Taibai Mountain, Shanxi, China; Li Q-Q 09080901; *A. membranaceum* Ownbey ex Traub EU096165[6]; *A. moly* L. AF055108[2]; *A. monanthum* Maxim. AJ412745[5]; *A. neapolitanum* Cirillo AF055104[2]; *A. obtusum* Lemmon EU096166[6]; *A. omeiense* Z. Y. Zhu 1—**GQ181076**; Emei Mountain, Sichuan, China; Xu J-M 91-01; *A. omeiense* Z. Y. Zhu 2—**HQ690567**; Emei Mountain, Sichuan, China; Hu H-Y em-20100626-2; *A. paradoxum* (M. Bieb.) G. Don AJ412741[5]; *A. parvum* Kellogg EU096169[6]; *A. peninsulare* Lemmon ex Greene var. *peninsulare* EU096170[6]; *A. platycaule* S. Watson EU096171[6]; *A. praecox* Brandegee EU096173[6]; *A. punctum* L. F. Hend. EU096174[6]; *A. roseum* L. AF055105[2]; *A. sanbornii* Alph. Wood var. *sanbornii* EU096177[6]; *A. serra* McNeal & Ownbey EU096178[6]; *A. sharsmithiae* (Ownbey & Aase) McNeal EU096179[6]; *A. shevockii* McNeal EU096180[6]; *A. siculum* Ucria AJ250299[3]; *A. siskiyouense* Munz & Keck ex Ownbey EU096181[6]; *A. stellatum* Nutt. ex Ker Gawl. EU096183[6]; *A. subhirsutum* L. AF055106[2]; *A. tribracteatum* Torr. EU096184[6]; *A. triquetrum* L. AJ412742[5]; *A. tuolumnense* (Ownbey & Aase) S. S. Denison & McNeal EU096185[6]; *A. unifolium* Kellogg EU096186[6]; *A. ursinum* L. AJ412744[5]; *A. validum* S. Watson EU096188[6]; *A. wallichii* Kunth var. *wallichii* 1—**HQ690253**; Wenbi Mountain, Lijiang, Yunan, China; Liu S & Gao P 20100903-02; *A. wallichii* Kunth var. *wallichii* 2—**HQ690251**; Hou shan, Yicun, Xianggelila, Yunan, China; Li Q-Q YC09072907; *A. wallichii* Kunth var. *wallichii* 3—**HQ690250**; Mountain near Potatso National Park, Xianggelila, Yunan, China; Li Q-Q BT09072818; *A. wallichii* Kunth var. *wallichii* 4—**GQ181091**; Kangding, Sichuan, China; Li Q-Q 2008081203; *A. wallichii* Kunth var. *wallichii* 5—**HQ690252**; Jilong, Xizang, China; Yu Y yy10080905; *A. wallichii* Kunth var. *wallichii* 6—**HQ690249**; Mahuangba, Lijiang, Yunan, China; Li Q-Q MH09072515; *A. wallichii* Kunth var. *wallichii* 7—**HQ690566**; Yulong Snow Mountain, Lijiang, Yunan, China; Li Q-Q YL09072630; *A. wallichii* var. *platyphyllum* (Diels) J. M. Xu—**GU566624**; Lijiang, Yunnan, China; Li Q-Q 09072503; *A. yosemitense* Eastw. EU096189[6]; *A. zebdanense* Boiss. & Noë AY427552[4]; Subgenus *Microscordum* (Maxim.) N. Friesen: *A. monanthum* Maxim. AJ412745[5]; Subgenus *Nectaroscordum* (Lindl.) Asch. et, Graebn.: *A. bulgaricum* (Janka) Prodan AJ412747[5]; *A. siculum* Ucria AJ250299[3]